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Colistin-resistant carbapenemase-producing isolates among *Klebsiella* spp. and *Acinetobacter baumannii* in Tripoli, Libya

Sir,

The emergence of acquired carbapenemases is a serious threat to public health worldwide, forcing the use of last-resort antibiotics such as polymyxins. Use of such molecules had recently led to the emergence of colistin-resistant carbapenemase-producing isolates, leaving only a few therapeutic options for the near future. Here we report the identification of colistin-resistant isolates among a collection carbapenemase-producing Enterobacteriaceae and *Acinetobacter baumannii* isolates.

A total of 61 imipenem-non-susceptible isolates were recovered from different clinical samples from patients in Tripoli Medical Centre (Tripoli, Libya) between 2014 and 2015. Among this collection, 32/61 were identified as *Klebsiella pneumoniae*, 28/61 as *A. baumannii* and 1/61 as *Klebsiella oxytoca* using API® gallery (bioMérieux, Marcy-l'Étoile, France).

Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) recommendations. Carbapenemase activity was determined using the RAPIDEC® CARBA NP test (bioMérieux) for Enterobacteriaceae and the modified CarbAcineto NP test for *A. baumannii*, showing positive results for all isolates. Resistance to colistin was determined with the Rapid Polymyxin™ NP test for Enterobacteriaceae [1]. Imipenem and colistin minimum inhibitory concentrations (MIC) were evaluated by Etest and broth microdilution, respectively. All isolates presenting a colistin MIC >2 μg/mL were considered resistant. All data are summarised in Table 1.

The resistance phenotype towards other families of antibiotics was determined using the standard disk diffusion method. All of the isolates presented a multidrug-resistant phenotype. Molecular investigations involved the detection of carbapenemases, 16S rRNA methylases and colistin resistance-related genes by PCR amplification using specific primers, followed by sequencing (Microsynth AG, Balgach, Switzerland). the 28 *A. baumannii* isolates were positive for the $bla_{\text{OXA-23}}$ gene and 12/28 were positive for the *armA* 16S rRNA methylase-encoding gene conferring high-level resistance to aminoglycosides. In addition, 5/28 isolates were resistant to colistin with an MIC between 4 μg/mL and 128 μg/mL with an unknown resistance mechanism.

Among the 33 carbapenem-resistant *Klebsiella* spp. isolates, 28/33 and 20/33 were positive by PCR for the $bla_{\text{OXA-48}}$ and $bla_{\text{NDM-1}}$ genes, respectively. Noteworthy, 15/33 isolates were positive for both carbapenemase genes. Six isolates were found to be resistant to colistin with an MIC ranging between 64 μg/mL and 128 μg/mL (Table 1). Noteworthy, the 16S rRNA methylase-encoding gene *rmtC* was detected in three *K. pneumoniae* isolates and one *K. oxytoca* isolate.

The clonal relationship of the *Klebsiella* and *Acinetobacter* isolates was evaluated by pulsed-field gel electrophoresis (PFGE). Briefly, total DNA from bacterial isolates was digested by *Xba*I or *Apa*I restriction enzymes (New England Biolabs, Ipswich, MA) for the *Klebsiella* and *Acinetobacter* isolates, respectively. The generated fragments were separated using a CHEF-DR® III System (Bio-Rad) and the different pulsotypes were identified using the MultiVariate Statistics Package (MVSP) software. All PFGE profiles showing a similarity coefficient >0.8 were assigned to the same cluster. Multilocus sequence typing (MLST) was performed for the colistin-resistant isolates and sequence types (STs) were assigned using the online databases http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html and https://pubmlst.org/bigsdb?db=pubmlst_a-baumannii_pasteur_seqdef. A total of 11 different pulsotypes were identified among the *K. pneumoniae* collection, whilst 12 pulsotypes were detected among the *Acinetobacter* collection (Table 1). The six colistin-resistant *K. pneumoniae* isolates carried both the $bla_{\text{OXA-48}}$ and $bla_{\text{NDM-1}}$ carbapenemase genes. MLST analysis revealed that these isolates belonged to ST101. Insertion of an insertion sequence (IS) identified as IS*903B* into the *mgrB* gene between nucleotides 107 and 108 for Kp65 and Kp82 isolates and insertion of an IS*1R* mobile element in the promotor region of the *mgrB* gene for Kp173, Kp174, Kp190 and Kp191 isolates (44 nucleotides before the GTG start codon) were detected by PCR amplification. These IS elements are likely to modify MgrB expression and to be responsible for the high-level colistin resistance observed in these isolates, as has been described previously [2]. PCR experiments did not reveal the presence of the *mcr*-like plasmid-mediated colistin resistance genes.

Monitoring the occurrence of multidrug-resistant bacteria is crucial to avoid the spread of such isolates in clinical facilities. Little information is known about the dissemination of carbapenemase-producers in Libya and only a few clinical cases of OXA-48-producing isolates recovered from Libyan refugees in Europe have been described [3,4]. However, more recently, an epidemiological study described a collection of NDM-1 and OXA-23 carbapenemase-producing *A. baumannii* in a Libyan hospital [5]. Here we described multiresistant *K. pneumoniae* and *A. baumannii* isolates. To the best of our knowledge, this is the first study describing colistin-resistant and 16S RNA methylase-producing isolates in that area. This study underlines that multidrug resistance, possibly leading to pandrug resistance, may be spreading under-recognised in that part of the world.

Table 1
Genotypic and phenotypic features of the collection of imipenem-non-susceptible *Acinetobacter baumannii* and *Klebsiella* spp. isolates.

Strain ID	Species	Pulsotype (MLST)	Sample	MIC (μg/mL)		Resistance gene(s)	Resistance phenotype
				IPM	COL		
A37	*Ab*	10 (ST2)	Sputum	>32	128	*armA*, bla_{OXA-23}	AMK/CHL/COL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A29	*Ab*	1 (ST164)	CSF	>32	32	bla_{OXA-23}	CHL/COL/CIP/GEN/IPM/TET/TOB
A11	*Ab*	3 (ST745)	Eye	>32	8	bla_{OXA-23}	CHL/COL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A13	*Ab*	4 (ND)	Pleural fluid	>32	4	bla_{OXA-23}	CHL/COL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A28	*Ab*	11 (ST164)	Sputum	>32	4	bla_{OXA-23}	CHL/COL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A31	*Ab*	3 (ST745)	ETT	>32	1	*armA*, bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A33	*Ab*	6 (ST2)	Blood	>32	2	*armA*, bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A34	*Ab*	8 (ST2)	Urine	>32	0.5	*armA*, bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A36	*Ab*	9 (ST2)	Central line	>32	1	*armA*, bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A76	*Ab*	8 (ST2)	Urine	>32	2	*armA*, bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A87	*Ab*	6 (ST2)	Ear	>32	0.5	*armA*, bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A19-a	*Ab*	5 (ST2)	Urine	>32	0.25	*armA*, bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A91	*Ab*	9 (ST2)	Swab	>32	0.5	*armA*, bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A92	*Ab*	9 (ST2)	Sputum	>32	0.25	*armA*, bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A23	*Ab*	5 (ST2)	Blood	>32	1	bla_{OXA-23}	CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A20-a	*Ab*	5 (ST2)	Wound	>32	1	*armA*, bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A32	*Ab*	7 (new ST)	Blood	>32	1	bla_{OXA-23}	CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A7	*Ab*	1 (ST164)	Blood	>32	0.5	bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/TET/TOB
A15	*Ab*	3 (ST745)	Eye	>32	0.5	bla_{OXA-23}	AMK/CHL/CIP/IPM/SUL/SXT/TET/TOB
A24	*Ab*	6 (ST2)	Blood	>32	0.5	bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A27	*Ab*	2 (ST164)	ETT	>32	0.5	bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/TET/TOB
A86	*Ab*	6 (ST2)	Central line	>32	0.5	bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A8	*Ab*	1 (ST164)	ETT	>32	0.25	bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
A17	*Ab*	2 (ST164)	UVC	>32	0.25	bla_{OXA-23}	CHL/CIP/GEN/IPM/SUL/TET/TOB
A35	*Ab*	1 (ST164)	CSF	>32	0.25	*armA*, bla_{OXA-23}	AMK/CIP/GEN/IPM/SUL/SXT/TET/TOB
A39	*Ab*	12 (ST164)	Swab	>32	0.25	bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/TET/TOB
A10	*Ab*	2 (ST164)	UVC	>32	0.125	bla_{OXA-23}	AMK/CHL/CIP/GEN/IPM/TET/TOB
A16	*Ab*	2 (ST164)	UVC	>32	0.125	bla_{OXA-23}	AMK/CIP/GEN/IPM/SUL/TET/TOB
Kp173	*Kp*	1 (ST101)	Pus	16	128	bla_{OXA-48}, bla_{NDM-1}	CIP/COL/GEN/IPM/SUL/SXT/TET/TOB
Kp174	*Kp*	1 (ST101)	Blood	16	64	bla_{OXA-48}, bla_{NDM-1}	CIP/COL/GEN/IPM/SUL/SXT/TET/TOB
Kp190	*Kp*	1 (ST101)	Blood	16	128	bla_{OXA-48}, bla_{NDM-1}	CIP/COL/GEN/IPM/SUL/TET/TOB
Kp191	*Kp*	1 (ST101)	Sputum	16	128	bla_{OXA-48}, bla_{NDM-1}	CIP/COL/GEN/IPM/SUL/TET/TOB
Kp65	*Kp*	2 (ST101)	Urine	2	64	bla_{OXA-48}, bla_{NDM-1}	AMK/CIP/COL/GEN/IPM/SXT/TET/TOB
Kp82	*Kp*	2 (ST101)	Swab	3	64	bla_{OXA-48}, bla_{NDM-1}	AMK/CIP/COL/GEN/IPM/SXT/TET/TOB
Kp50	*Kp*	11 (ST15)	Sputum	3	0.125	bla_{OXA-48}, bla_{NDM-1}, *rmtC*	AMK/CIP/GEN/IPM/SUL/SXT/TOB
Kp69	*Ko*	9 (ND)	Central line	8	0.125	bla_{OXA-48}, bla_{NDM-1}, *rmtC*	AMK/GEN/IPM/SUL/TOB
Kp93	*Kp*	3 (ST101)	ETT	3	0.125	bla_{OXA-48}, bla_{NDM-1}, *rmtC*	AMK/CIP/GEN/IPM/SUL/SXT/TET/TOB
Kp42	*Kp*	8 (ST147)	Wound	1	0.125	bla_{OXA-48}, bla_{NDM-1}	CHL/CIP/GEN/IPM/TET/TOB
Kp51	*Kp*	11 (ST15)	Urine	>32	0.125	bla_{OXA-48}, bla_{NDM-1}, *rmtC*	AMK/CIP/GEN/IPM/SUL/SXT/TOB
Kp53	*Kp*	7 (ST147)	Swab	1	0.125	bla_{OXA-48}, bla_{NDM-1}	CIP/GEN/IPM/SUL/SXT/TET/TOB
Kp58	*Kp*	7 (ST147)	Urine	1	0.125	bla_{OXA-48}, bla_{NDM-1}	CIP/GEN/IPM//TET/TOB
Kp61	*Kp*	10 (ST11)	ETT	>32	0.125	bla_{OXA-48}, bla_{NDM-1}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TET/TOB
Kp52	*Kp*	7 (ST147)	Blood	1	0.125	bla_{OXA-48}, bla_{NDM-1}	CIP/GEN/SUL/SXT/TET/TOB
Kp59	*Kp*	10 (ST11)	Catheter	>32	0.125	bla_{NDM-1}	AMK/CHL/CIP/GEN/IPM/SUL/SXT/TOB
Kp60	*Kp*	10 (ST11)	Swab	>32	0.125	bla_{NDM-1}	AMK/CIP/GEN/IPM/SUL/SXT/TOB
Kp78	*Kp*	2 (ST101)	Urine	>32	0.125	bla_{NDM-1}	CIP/GEN/IPM/SUL/TET/TOB
Kp94	*Kp*	7 (ST147)	Nose	0.5	0.125	bla_{NDM-1}	CIP/GEN/SUL/SXT/TET/TOB
Kp12	*Kp*	5 (ST405)	Wound	1	0.125	bla_{NDM-1}	CIP/GEN/IPM/SUL/SXT/TET/TOB
Kp25	*Kp*	2 (ST101)	Wound	3	0.125	bla_{OXA-48}	CIP/GEN/IPM/SXT/TET/TOB
Kp88	*Kp*	5 (ST405)	Blood	0.75	0.125	bla_{OXA-48}	CIP/GEN/SUL/SXT/TET/TOB
Kp89	*Kp*	5 (ST405)	ETT	0.75	0.125	bla_{OXA-48}	CIP/GEN/SUL/SXT/TET/TOB
Kp84	*Kp*	2 (ST101)	ETT	0.38	0.125	bla_{OXA-48}	CIP/GEN/SUL/SXT/TET/TOB
Kp95	*Kp*	3 (ST101)	ETT	0.38	0.125	bla_{OXA-48}	CIP/GEN/TET/TOB
Kp49	*Kp*	8 (ST147)	Sputum	0.5	0.125	bla_{OXA-48}	AMK/CIP/GEN/SUL/SXT/TET/TOB
Kp56	*Kp*	6 (ST405)	ETT	0.5	0.125	bla_{OXA-48}	CIP/GEN/SUL/SXT/TET/TOB
Kp57	*Kp*	6 (ST405)	Blood	0.5	0.125	bla_{OXA-48}	CIP/GEN/SUL/SXT/TET/TOB
Kp70	*Kp*	6 (ST405)	Blood	0.5	0.125	bla_{OXA-48}	CIP/GEN/SUL/SXT/TET/TOB
Kp72	*Kp*	5 (ST405)	ETT	0.5	0.125	bla_{OXA-48}	CIP/GEN/SUL/SXT/TET/TOB
Kp80	*Kp*	4 (ST45)	ETT	0.5	0.125	bla_{OXA-48}	GEN/SXT/TET/TOB
Kp71	*Kp*	2 (ST101)	Blood	0.5	0.125	bla_{OXA-48}	CIP/GEN/TET/TOB
Kp85	*Kp*	3 (ST101)	ETT	0.5	0.125	bla_{OXA-48}	CIP/GEN/SUL/SXT/TET/TOB

Ab, *Acinetobacter baumannii*; *Kp*, *Klebsiella pneumoniae*; *Ko*, *Klebsiella oxytoca*; MLST, multilocus sequence typing; ND, not determined; CSF, cerebrospinal fluid; ETT, endotracheal tube; UVC, umbilical vein catheter; MIC, minimum inhibitory concentration; IPM, imipenem; COL, colistin; AMK, amikacin; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; SUL, sulfonamide; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; TOB, tobramycin.

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Competing interests

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Ethical approval

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